$C_{21}H_{29}NO_5$: C, 67.2; H, 7.8; N, 3.7. Found: C, 67.2; H, 7.8; N, 3.9], characterized as the **acetate** (m.p. 206–208°; reported[6] m.p. 210°.)

Reduction of the carbonyl group in tetrahydrodemethoxycolchicine to methylene was effected by preparing the **dimethylmercaptol** [m.p. 190°; $[\alpha]^{25}D$ −160° (*c*, 0.96, ethanol); *Anal.* Calcd. for $C_{23}H_{33}NO_4S_2$: C, 61.2; H, 7.4; S, 14.2. Found: C, 61.4; H, 7.5; S, 14.0] which on heating with Raney nickel gave hexahydrodemethoxydesoxycolchicine[7] [m.p. 183.5–184°; $[\alpha]^{25}D$ −162° (*c*, 1.10, ethanol); *Anal.* Calcd. for $C_{21}H_{29}NO_4$: C, 70.2; H, 8.1; OCH_3, 25.9. Found: C, 70.1; H, 8.2; OCH_3, 26.0]. Titration with perbenzoic acid showed the presence of 1.07 double bonds.

Phosphorus pentoxide in refluxing xylene degraded hexahydrodemethoxydesoxycolchicine to the desacetamido compound which was directly hydrogenated (1.1 moles of hydrogen absorbed) to octahydrodemethoxydesoxydesacetamidocolchicine (II) [m.p. 49–50°; $[\alpha]^{25}D$ 0° (*c*, 1.01, ethanol); *Anal.* Calcd. for $C_{19}H_{26}O_3$: C, 75.5; H, 8.7; OCH_3, 30.8. Found: C, 75.4; H, 8.7; OCH_3, 30.9]. Titration with perbenzoic acid showed the presence of 1.02 double bonds and gave a crystalline *oxide* (m.p. 115–116°; *Anal.* Calcd. for $C_{19}H_{26}O_4$: C, 71.7; H, 8.2. Found: C, 71.6; H, 8.3). The ultraviolet and infrared absorption spectra of the various degradation products above were determined and found to be compatible with the assigned structures.

DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA
HENRY RAPOPORT
ARTHUR R. WILLIAMS[8]

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(7) A compound of this empirical formula and m.p. 182–183° was isolated in small amounts by Bursian (ref. 5) from the hydrogenation of colchicine. However, Kemp and Tarbell (ref. 6) reported none of this material among the hydrogenation products of colchicine.

(8) American Cancer Society Postdoctoral Fellow.

COMPARISON OF CITROVORUM FACTOR AND A SYNTHETIC COMPOUND WITH LEUCONOSTOC CITROVORUM GROWTH ACTIVITY

Sir:

By the application of purification procedures to desiccated liver powder we have obtained concentrates of the citrovorum factor (I) with substantially the same activity, weight for weight, for *Leuconostoc citrovorum* 8081 as the recently reported compound resulting from the formylation and reduction of pteroylglutamic acid[1] (II). The differences in the microbiological activity of the respective acid degradation products and the absorption spectra lead us to believe that the synthetic compound[2] is not the citrovorum factor as obtained from liver.

The method used for the preparation of our concentrates was based on that previously described,[3] extended and modified to include Florisil and Dowex 1 chromatograms. The use of barium and silver precipitations was eliminated. The product (I) which was obtained by fractional precipitation from methanol of eluates from Al_2O_3 columns was found by assay with *Leuconostoc citrovorum* 8081 to contain 176 CF units[3] per γ. Under the same assay conditions II contained 152 units per γ.

When assayed for folic acid activity using *Streptococcus faecalis* R, 1γ of I was equivalent to 0.648 γ pteroylglutamic acid (PGA) while 1γ of II had a value of 0.572γ PGA. When stored at *p*H 2.0, for 20 hours at 23°, both materials showed 96–97% loss of citrovorum activity. However, as is the case with much cruder materials,[3] I exhibited 32% loss of PGA activity when assayed with *Streptococcus faecalis* R; on the other hand, II showed an enhanced PGA activity of approximately 13%. This increase in activity was found consistently and could not be ascribed to errors inherent in the microbiological assay.

While both materials in 30% ethanol containing 0.03% NH_3 showed a maximum at approximately the same wave length, there was a very marked difference in the intensity. At a concentration of 10 mg/l. I exhibited 38.6% T at 286 mμ as compared with 24.6% T at the same wave length for II. Assuming both I and II have the same chromophoric group and if there is no great difference in their molecular weights, then I can be calculated to have a purity of approximately 70%.

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, FEDERAL SECURITY AGENCY
BETHESDA 14, MARYLAND
MILTON SILVERMAN
JOHN C. KERESZTESY

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L-HISTIDINOL, A PRECURSOR OF L-HISTIDINE IN *Escherichia coli*

Sir:

Of three mutant strains of *Escherichia coli* responding to histidine, one (26-25) excretes a substance that satisfies the histidine requirement of the other two (26-24, 26-24D1). Strain 26-24D1 was derived from 26-24 and differs from it by more rapid utilization of the excreted substance. The latter was isolated from culture filtrates of 26-25 by adsorption on charcoal (Darco G-60) at *p*H 7.5, elution with decinormal hydrochloric acid in 85% ethanol, evaporation to dryness of the eluate, and precipitation with picric acid from aqueous solution. The resulting dipicrate, recrystallized from water (m.p. 194–197°. *Anal.* Calcd. for $C_{18}H_{17}O_{15}N_9$: C, 36.07; H, 2.86; N, 21.03. Found: C, 36.19; H, 3.03; N, 20.98), was treated with normal hydrochloric acid and the liberated picric acid removed with ether. On evaporating the aqueous phase to dryness, the active material (85 to 150 mg. per liter culture filtrate) was obtained as dihydrochloride and recrystallized twice from 95% ethanol. It sinters at 193° and melts at 197–199.5° on the micro-block, $[\alpha]^{20}D$ −3.0° (*c*, 5.0 in water). *Anal.* Calcd. for $C_6H_{13}ON_3Cl_2$: C, 33.66; H, 6.12; N, 19.63. Found: C, 33.67; H, 6.00; N, 19.53. These data, together with the fact that the dihydrochloride could be oxidized to L-histidine, indicated

(1) Brockman, Roth, Broquist, Hultquist, Smith, Fahrenbach, Cosulich, Parker, Stokstad and Jukes, THIS JOURNAL, **72**, 4325 (1950).

(2) Samples of crystalline free acid kindly supplied by Dr. Thomas H. Jukes, Lederle Labs. Division, American Cyanamid Company, Pearl River, N. Y.

(3) Keresztesy and Silverman, *J. Biol. Chem.*, **183**, 473 (1950).